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Preview

Tandem Modifications of an Epoxyquinone C_7N Pharmacophore

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C_7N moieties are wildly present as pharmacophores in natural products. In this issue of *Chemistry & Biology*, Rui and colleagues biochemically reproduced the initiation event of asukamycin biosynthesis and characterized tandem enzymatic oxygenations of the epoxyquinone C_7N moiety.

Many families of microbial secondary metabolites owe their diverse bioactivities to the presence of pharmacologically active C_7N moieties. These C_7N moieties are present in various structural forms and synthesized through different enzymatic routes (Figure 1). The large family of ansamycins, such as the anti-tuberculosis rifamycin and antitumor ansamitocin, use 3-amino-5-hydroxybenzoic acid (3,5-AHBA) as the starter unit to initiate the polyketide chain assembly en route to the C_7N pharmarcophore. 3,5-AHBA is synthesized from UDP-glucose and glutamine by a dedicated set of enzymes through a route similar to the shikimate pathway (Figure 1A) (Floss, et al., 2011). So far, the reported modifications that occur at the 3,5-AHBA moieties of ansamycins include quino reduction and glycolate unit addition (rifamycin), halogenation and *O*-methylation (ansamitocin), and hydroxylation (rubradirin) (Kang, et al., 2012).

C_7N moieties are also found in the family of aminocylitol natural products exemplified by the antidiabetic acarbose and antifungal validamycin. The C_7N units are derived from the pentose phosphate pathway with sedo-heptulose 7-phosphate as the immediate precursor to generate 2-*epi*-5-*epi*-valiolone, which subsequently undergoes further modifications to produce the C_7N moieties with significant structural variations (Figure 1B). These enzymatic modifications include epimerization, dehydration, glycosylation, hydroxylation, and epoxydation (Flatt and Mahmud, 2007).

The manumycin-type metabolites, including manumycin and asukamycin, are a new family of C_7N-containing natural products that display antibacterial, anticoccidal, antifungal, and other attractive activities. This type of compound shows unique features with an epoxyquinone meta-C_7N moiety, a "lower" polyketide chain, an "upper" polyketide chain, and a C_5N ring linked to the "lower" chain (Sattler, et al., 1998). Previous intensive synthetic and feeding experiments done by Floss and coworkers had established 3-amino-4-hydroxybenzoic acid (3,4-AHBA) as the putative starter unit for the "lower" chain assembly and protoasukamycin as a true late intermediate (Figure 1C) (Hu and Floss, 2004). Recently, Rui et al. (2010) reported the cloning of the 63.9 kb asukamycin biosynthetic gene cluster and performed a systematic inactivation of more than 17 genes, leading to a more rational asukamycin biosynthetic pathway.

Similar to the biosynthetic studies of other natural products, the in vivo gene inactivation usually could not tell the exact functions of each gene and especially the order or timing of each reaction in the biosynthetic pathway. In this issue of *Chemistry & Biology*, using elegant enzymatic catalysis coupled with careful LC-HRMS analysis, Rui et al. (2013) clearly deciphered the process of 3,4-AHBA activation and initiation of the "lower" chain assembly and the tandem oxygenations involved in the epoxyquinone C_7N formation.

In the asukamycin biosynthetic gene cluster, *asuA2* is the only gene coding for adenylase and is considered to be critical for the activation of 3,4-AHBA. Using the classical ATP-[^{32}P]PP*i* exchange assay, 3,4-AHBA and other four aryl acids were found to be efficiently adenylated by the heterologously expressed AsuA2, reflecting its catalytic promiscuity. Moreover, the two candidate ACPs, AsuC11 and AsuC12, within the cluster were expressed in holo forms and tested to identify the cognate ACP for AsuA2. Detected by LC-HRMS, AsuC12 was shown to be the true receptor for 3,4-AHBA and other four aryl acids, whereas no reaction occurred with AsuC11. However, *asuC11* was previously shown to be essential for asukamycin biosynthesis through gene inactivation, suggesting that AsuC11 may have other functions rather than being an acyl-carrier protein.

The putative hydroxylase AsuE1 converted protoasukamycin to 4-hydroxyprotoasukamycin, a known intermediate of asukamycin biosynthesis, in the presence of NADPH and abundant flavin. Under this circumstance, AsuE1 shows features of single-component flavin-dependent monooxygenase by equivalently reducing FAD, FMN, or riboflavin. However, the presence of AsuE2, a cognate flavin reductase, can replace the exogenous flavin and requires only the presence of bound FMN for the hydroxylation by AsuE1, highlighting the two-component nature of AsuE1. From 4-hydroxyprotoasukamycin to the final asukamycin with the epoxyquinone C_7N moiety, an epoxidation at the C5-C6 position of 3,4-AHBA is required. In vitro biochemical catalysis not only confirmed that AsuE3 is the required epoxidase with FMN as preferred flavin, but also showed its stringent substrate specificity for 4-hydroxyprotoasukamycin. Moreover, AsuE2 does not change the catalytic efficiency of AsuE3, showing that AsuE3 is a single-component epoxidase.

The authors took a mutasynthesis approach and fed the four aryl acids

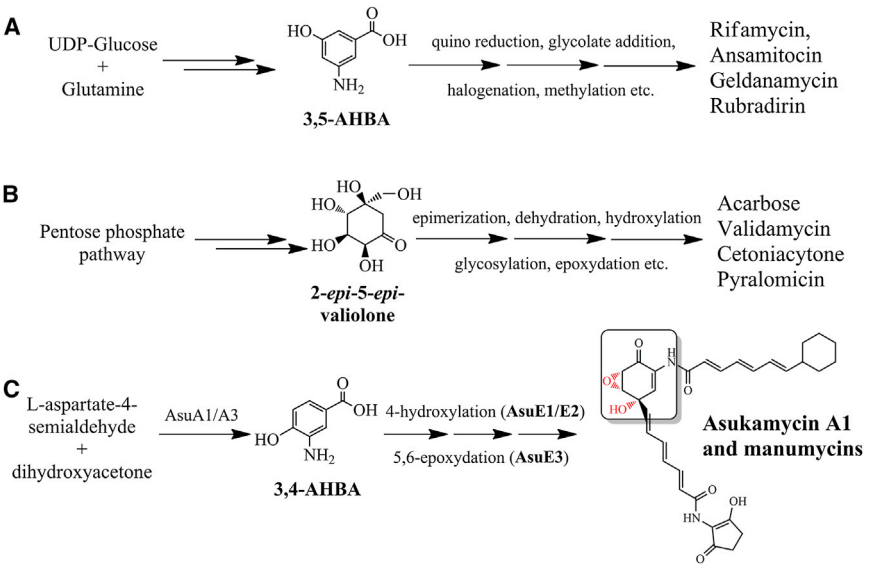

Figure 1. Three Families of Natural Products with Different C_7N Moieties
(A) Ansamycin-type natural products with 3,5-AHBA C_7N moiety.
(B) Aminocyclitol-type natural products with 2-*epi*-5-*epi*-valiolone as intermediate.
(C) Manumycin-type natural products with epoxyquinone C_7N moiety. The epoxyquinone moiety is highlighted with the square.

recognized by AsuA2 to a 3,4-AHBA nonproducing mutant. However, asukamycin analogs with intact structures were only identified in feeding experiment with 3-aminobezonic acid (3-ABA), suggesting the presence of highly specific and ordered catalytic activities in "upper" chain attachment and incorporation of the C_5N unit. Further structure characterization of 3-ABA derivatives actually proved that hydroxylation or epoxidation does not occur at the novel 3-ABA C_7N moiety, showing again the stringent substrate specificity of AsuE1/E3. Interestingly, all 3-ABA derivatives lost inhibitory activity against methicillin-resistant *S. aureus*, validating epoxyquinone as a pharmacologically active C_7N moiety.

Together with previous chemical and genetic achievements, the biochemical analysis carried out by Rui et al. (2013) disclosed details on the biosynthesis of manumycin-type antibiotics, which paves the way for further structure/activity modification and sheds new lights on improving the fermentation titers of these important drug leads.

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REFERENCES

Flatt, P.M., and Mahmud, T. (2007). Nat. Prod. Rep. *24*, 358–392.

Floss, H.G., Yu, T.W., and Arakawa, K. (2011). J. Antibiot. *64*, 35–44.

Hu, Y., and Floss, H.G. (2004). J. Am. Chem. Soc. *126*, 3837–3844.

Kang, Q., Shen, Y., and Bai, L. (2012). Nat. Prod. Rep. *29*, 243–263.

Rui, Z., Petrícková, K., Skanta, F., Pospísil, S., Yang, Y., Chen, C.Y., Tsai, S.F., Floss, H.G., Petrícek, M., and Yu, T.W. (2010). J. Biol. Chem. *285*, 24915–24924.

Rui, Z., Sandy, M., Jung, B., and Zhang, W. (2013). Chem. Biol. *20*, this issue, 879–887.

Sattler, I., Thiericke, R., and Zeeck, A. (1998). Nat. Prod. Rep. *15*, 221–240.

